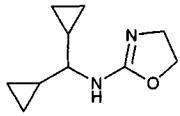
Rilmenidine



Molecular formula: $C_{10}H_{16}N_2O$
Molecular weight: 180.25
CAS Registry No.: 54187-04-1
Merck Index: 8388

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 9.8

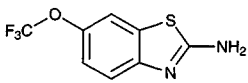
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Riluzole



Molecular formula: $C_8H_5F_3N_2OS$
Molecular weight: 234.20
CAS Registry No.: 1744-22-5
Merck Index: 8389

SAMPLE

Matrix: blood
Sample preparation: Add 100-200 ng IS to 1 mL plasma adjusted to pH 6.0. Vortex, centrifuge at 2000 g for 5 min. Add to a 1 mL C2 Bond Elut SPE cartridge or CBA Bond Elut ion exchange SPE cartridge. Wash with 1 mL 10 mM dipotassium hydrogen phosphate, 500 µL water, and MeOH:water 10:90. Elute with 500 µL mobile phase, dry under a stream of nitrogen. Inject a 300 µL aliquot.

HPLC VARIABLES
Guard column: 39 × 4.6 μBondapak C18
Column: 300 × 4.6 μBondapak C18
Mobile phase: MeCN:MeOH:acetic acid:dipotassium hydrogen phosphate 10:55:1:35
Flow rate: 1
Injection volume: 300
Detector: UV 265

CHROMATOGRAM
Retention time: 9.2
Internal standard: RP 61307 (riluzole N-methyl derivative) (12.4)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Le Liboux,A.; Lefebvre,P.; Le Roux,Y.; Truffinet,P.; Aubeneau,M.; Kirkesseli,S.; Montay,G. Single- and multiple-dose pharmacokinetics of riluzole in white subjects, *J.Clin.Pharmacol.*, **1997**, *37*, 820–827.

SAMPLE
Matrix: urine
Sample preparation: Add 20 μg IS to 1 mL urine adjusted to pH 6.0. Vortex, centrifuge at 2000 g for 5 min. Add to a 1 mL C2 Bond Elut SPE cartridge or CBA Bond Elut ion exchange cartridge. Wash with 1 mL 10 mM dipotassium hydrogen phosphate, 500 μL water, MeOH: water 10:90. Elute with 500 μL mobile phase, inject a 300 μL aliquot.

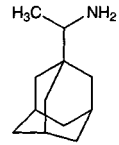
HPLC VARIABLES
Guard column: 39 × 4.6 μBondapak C18
Column: 300 × 4.6 μBondapak C18
Mobile phase: MeCN:MeOH:acetic acid:dipotassium hydrogen phosphate 10:55:1:35
Flow rate: 0.8-1
Injection volume: 300
Detector: UV 265

CHROMATOGRAM
Retention time: 9.2
Internal standard: RP 61 307 (riluzole N-methyl derivative) (12.4)
Limit of detection: 10 ng/mL

KEY WORDS
pharmacokinetics; SPE

REFERENCE
Le Liboux,A.; Lefebvre,P.; Le Roux,Y.; Truffinet,P.; Aubeneau,M.; Kirkesseli,S.; Montay,G. Single- and multiple-dose pharmacokinetics of riluzole in white subjects, *J.Clin.Pharmacol.*, **1997**, *37*, 820–827.

Rimantadine



Molecular formula: $C_{12}H_{21}N$
Molecular weight: 179.31
CAS Registry No.: 13392-28-4, 1501-84-4 (HCl)
Merck Index: 8390
Lednicer No.: 2 19

SAMPLE
Matrix: solutions

Sample preparation: 50 μL 5 mg/mL Rimantadine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 36.4 mg 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:buffer 85:15 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 420 or UV 254

CHROMATOGRAM

Retention time: 5.88, 7.11 (enantiomers)
Limit of detection: 6 ng (UV)

KEY WORDS

derivatization; protect from light; chiral

REFERENCE

Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE

Matrix: urine
Sample preparation: Adjust pH of 8 mL urine to 5.5 with 2 M HCl, add 80 μL Glusulase (DuPont), heat at 37° for 18 h. Remove a 2 mL aliquot and add it to 500 μL 5 M NaOH, add 8 mL cyclohexane saturated with triethanolamine:chloroform 2:1, add 100 μL 2% pentafluorobenzoyl chloride in cyclohexane, shake at 30 strokes/min for 20 min, centrifuge at 1500 g. Remove 7.5 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 160 μL heptane:isopropanol 95:5, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax silica
Mobile phase: Gradient. Hexane:isopropanol 95:5 for 10 min, to 90:10 over 20 min, re-equilibrate for 5 min.
Flow rate: 1.5
Detector: radioactivity

CHROMATOGRAM

Retention time: 6.4

OTHER SUBSTANCES

Extracted: metabolites

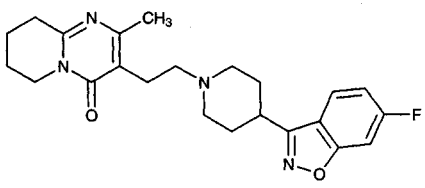
KEY WORDS

14C-labeled; derivatization; normal phase

REFERENCE

Rubio,F.R.; Fukuda,E.K.; Garland,W.A. Urinary metabolites of rimantadine in humans, *Drug Metab.Dispos.*, **1988**, *16*, 773–777.

Risperidone



Molecular formula: $C_{23}H_{27}FN_4O_2$
Molecular weight: 410.49
CAS Registry No.: 106266-06-2
Merck Index: 8397
Lednicer No.: 5 150

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 500 μL 600 mM pH 10 sodium carbonate/bicarbonate buffer + 50 μL 3.76 mg/mL IS in MeOH + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min. Freeze the aqueous layer, evaporate the heptane layer to dryness under a gentle stream of nitrogen at 60°. Dissolve the residue in 75 μL mobile phase, inject a 65 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 LiChroCart
Mobile phase: MeOH:40 mM pH 7.0 ammonium acetate buffer 90:10
Flow rate: 1
Injection volume: 65
Detector: UV 280

CHROMATOGRAM

Retention time: 4.42
Internal standard: haloperidol (5.10)
Limit of quantitation: 1.2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites IN clozapine, diltiazem, fluphenazine, hydroxyzine, mianserine, perphenazine, zuclopenthixol
Simultaneous: amitriptyline, citalopram, chlorprothixene, clomipramine, desipramine, desmethylcitalopram, desmethylclomipramine, desmethylsertraline, fluoxetine, 10-hydroxyamitriptyline, 8-hydroxyclomipramine, 8-hydroxydesmethylclomipramine, 10-hydroxynortriptyline, imipramine, methotrimeprazine sulfoxide, norfluoxetine, nortriptyline, paroxetine, sertraline
Noninterfering: carbamazepine, clonazepam, flunitrazepam, nitrazepam, oxazepam, oxcarbazepine

KEY WORDS

serum

REFERENCE

Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotropic drugs, *J.Chromatogr.B*, **1997**, *698*, 209–216.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 25 ng/mL remoxipride in MeCN, mix for 5 s, add 1 mL saturated sodium carbonate, mix for 5 s, add 7 mL pentane:dichloromethane 75:25, shake gently for 10 min, centrifuge at 18° at 1735 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 140 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:MeOH:40 mM pH 6.8 ammonium acetate 82:8:10
Column temperature: 40
Flow rate: 1.5

Injection volume: 150
Detector: E, ESA Coulochem model 5100A, model 5011 analytical cell, screening electrode 0.6 V, detection electrode 0.92 V, model 5020 guard cell 1 V

CHROMATOGRAM
Retention time: 14
Internal standard: remoxipride (17)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: pseudoephedrine
Noninterfering: metabolites, acetaminophen, benztropine, clonazepam, clozapine, fluphenazine, haloperidol, ibuprofen, lorazepam, trihexyphenidyl

KEY WORDS
plasma

REFERENCE
Aravagiri,M.; Marder,S.R.; Van Putten,T.; Midha,K.K. Determination of risperidone in plasma by high-performance liquid chromatograhy with electrochemical detection: application to therapeutic drug monitoring in schizophrenic patients, *J.Pharm.Sci.*, **1993**, *82*, 447–449.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane: dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 7.8
Internal standard: methylrisperidone (R68808) (14.3)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, imipramine, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid
Interfering: pipamperone

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Urine. Inject up to 1.95 mL urine directly. Feces. Homogenize (Ultra-Turrax TP-25) feces with MeOH, centrifuge, repeat extraction twice more. Evaporate a 10 mL aliquot of the extracts to dryness under a stream of nitrogen, reconstitute in DMSO, inject a 200 μL aliquot. Plasma. Add an equal volume of MeCN to plasma, centrifuge. Remove the supernatant and reduce the volume under a stream of nitrogen at 40°, inject a 2 mL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Hypersil C18
Mobile phase: Gradient. A was 100 mM ammonium acetate containing 0.2% diethylamine, pH 6.0. B was MeCN:MeOH:1 M ammonium acetate containing 2% diethylamine, pH 6.0 80:10:10. A:B 100:0 to 50:50 over 1 h, maintain at 50:50 for 5 min, to 0:100 over 5 min.
Injection volume: 200-2000
Detector: UV 280 or radioactivity

CHROMATOGRAM
Retention time: 49

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Mannens,G.; Huang,M.-L.; Meuldermans,W.; Hendrickx,J.; Woestenborghs,R.; Heykants,J. Absorption, metabolism, and excretion of risperidone in humans, *Drug Metab.Dispos.*, **1993**, *21*, 1134–1141.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Plasma, urine. 1 mL Plasma or urine + 200 μL MeOH + 100 μL 2 μg/mL IS in MeOH + 1 mL 50 mM sodium borate, add 4 mL ethyl acetate, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and add them to 3 mL 50 mM sulfuric acid, extract. Add the aqueous layer to 150 μL concentrated ammonia, extract twice with 2.5 mL portions of heptane:isoamyl alcohol 90:10. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 80 μL mobile phase, inject a 30 μL aliquot. Tissue. Grind (Waring blender) tissue, homogenize (Ultra-Turrax) with 4 volumes of water. 1 mL Homogenate + 100 μL 2 μg/mL IS in MeOH + 1 mL 100 mM sodium borate, add 4 mL ethyl acetate, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and add them to 3 mL 50 mM sulfuric acid, extract. Add the aqueous layer to 150 μL concentrated ammonia, extract twice with 2.5 mL portions of heptane:isoamyl alcohol 90:10. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 80 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5 μm ODS-Hypersil
Mobile phase: MeCN:water:diethylamine 35:65:0.02
Flow rate: 0.8
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 2.9
Internal standard: 3-[2-[4-([6-fluoro-1,2-benzisoxazol-3-yl]methyl)-1-piperidinyl]ethyl]-2,7-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one (Janssen R 68808) (5.4)
Limit of quantitation: 10 ng/g (tissue), 2 ng/mL (plasma, tissue)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: diazepam, oxazepam
Interfering: desmethyldiazepam

KEY WORDS
plasma; dog; human; muscle; pharmacokinetics

REFERENCE
Woestenborghs,R.; Lorreyne,W.; Van Rompaey,F.; Heykants,J. Determination of risperidone and 9-hydroxyrisperidone in plasma, urine and animal tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *583*, 223–230.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

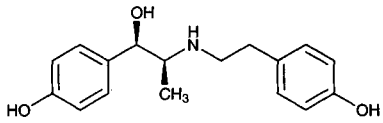
CHROMATOGRAM
Retention time: 9.12 (A), 4.63 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Ritodrine



Molecular formula: $C_{17}H_{21}NO_3$
Molecular weight: 287.36
CAS Registry No.: 26652-09-5, 23239-51-2 (HCl)
Merck Index: 8401
Lednicer No.: 2 39

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL water + 1 mL 600 mM potassium carbonate + 8 mL ethyl acetate, shake mechanically for 5 min, centrifuge at 2000 rpm for 15 min. Remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 5 min, centrifuge at 2000 rpm for 10 min. Remove the aqueous layer and add it to 500 μL 600 mM potassium carbonate and 1 mL ethyl acetate, shake for 5 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 5 μm Biophase ODS
Mobile phase: MeCN:buffer:water 20:10:70 containing 3 mM sodium 1-heptanesulfonate, pH 3.7 (Buffer was 2.1 M acetic acid containing 400 mM ammonium acetate.)
Flow rate: 1
Injection volume: 50
Detector: E, Bioanalytical Systems Model 4B, glassy carbon electrode 0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 9.33
Internal standard: nalbuphine (13.58)
Limit of detection: 0.6 ng/mL

KEY WORDS
plasma

REFERENCE
Kuhnert,P.; Erhard,P.; Dixon,A.; Kuhnert,B.; Gross,T. Determination of ritodrine in plasma using HPLC, *J.Liq.Chromatogr.*, **1983**, *6*, 2775–2783.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of 1 mL serum to 9.4 with 2 M sodium carbonate buffer, add 200 ng IS, vortex, add 6 mL ethyl acetate, shake for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 400 μL mobile phase, vortex vigorously, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 71 × 1.2 HC Pellosil (Whatman)
Column: 260 × 4.6 μBondapak C18
Mobile phase: MeOH:buffer 25:75 (Buffer was 10 mM KH_2PO_4 containing 0.3 mM sodium octanesulfonate and 0.1 mM EDTA, pH 4.5.)
Flow rate: 1.5
Injection volume: 40
Detector: E, Bioanalytical Systems Model LC-4B, glassy carbon electrode +0.90 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7.8
Internal standard: 1-(3,5-dihydroxyphenyl)-2-(1,1-dimethylbutylamino)ethanol (12.0)

Limit of detection: 0.2 ng

KEY WORDS
serum

REFERENCE
Lin,L.S.; Caritis,S.N.; Wong,L.K. Analysis of ritodrine in serum by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1984**, *73*, 131–133.

SAMPLE
Matrix: blood
Sample preparation: Dilute blood with an equal volume of water. 1 mL Plasma or 900 μL diluted blood + 50 μL 1.6 μg/mL isoxsuprine hydrochloride + 0.9-1 mL buffer + 5 mL freshly distilled ethyl acetate, vortex for 1 min, centrifuge at 1750 g for 7 min. Remove the organic layer and evaporate it almost to dryness under a stream of nitrogen at 57°, evaporate the final 500 μL at room temperature, reconstitute the residue in 100 μL MeCN, vortex for 15 s, inject the whole amount. (Buffer was 26.5 g sodium carbonate and 21 g sodium bicarbonate in 500 mL water, pH 9.48.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl (plasma) or 200 × 4.6 5 μm Spheri-5 RP-18 (blood)
Mobile phase: MeCN:0.05% orthophosphoric acid 17:83 (plasma) or 63:37 (blood)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 no emission filter or UV 254

CHROMATOGRAM
Retention time: 3.7 (plasma), 9.3 (blood)
Internal standard: isoxsuprine hydrochloride (15.1 (plasma), 16.3 (blood))
Limit of detection: 1 ng/mL (F)

OTHER SUBSTANCES
Simultaneous: fenoterol
Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, caffeine, chloral hydrate, dexamethasone, diazepam, lignocaine, meperidine, metoclopramide, morphine, nitrazepam, terbutaline

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Gross,A.S.; Brown,K.F.; Baird-Lambert,J.A.; Nation,R.L. Determination of ritodrine in blood and plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *416*, 400–408.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 225

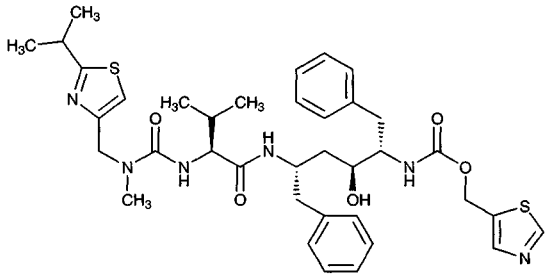
CHROMATOGRAM
Retention time: 3.43
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Ritonavir

Molecular formula: $C_{37}H_{48}N_6O_5S_2$
Molecular weight: 720.96
CAS Registry No.: 155213-67-5
Merck Index: 8402



SAMPLE
Matrix: bile, blood, feces, urine

Sample preparation: Rat, dog plasma. Add 3 volumes MeCN to plasma, vortex, centrifuge, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, filter (0.45 μm) while centrifuging, inject an aliquot. Human plasma. Add 3 volumes MeCN to plasma, vortex, centrifuge, rinse the protein pellet with MeCN and MeOH. Evaporate the combined supernatants to dryness under a stream of nitrogen, reconstitute the residue with 200 μL MeCN:MeOH:25 mM ammonium acetate 15:15:70 adjusted to pH 4.8 with trifluoroacetic acid, inject an aliquot. Feces. Centrifuge fecal homogenate, rinse the pellet twice with EtOH, filter the supernatants (0.45 μm), inject an aliquot. Bile. Filter bile and inject an aliquot. Urine. Concentrate urine under a stream of nitrogen or via lyophilization, reconstitute in mobile phase, filter while centrifuging, inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Nucleosil C18
Column: 250 × 4.6 5 μm Beckman Ultrasphere C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid adjusted to pH 4.8 with ammonium acetate. B was MeCN. A:B from 75:25 to 40:60 over 50 min
Flow rate: 1
Detector: A UV 220; B Radioactivity, Flo-One/Beta Model A-500 radioactivity with 0.5 mL flow cell

---

CHROMATOGRAM
Retention time: 43
Limit of detection: 150 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; rat; dog; human; radiolabeled

REFERENCE
Denissen,J.F.; Grabowski,B.A.; Johnson,M.K.; Buko,A.M.; Kempf,D.J.; Thomas,S.B.; Surber,B.W. Metabolism and disposition of the HIV-1 protease inhibitor ritonavir (ABT-538) in rats, dogs, and humans, *Drug Metab.Dispos.*, **1997**, *25*, 489–501.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL of 500 mM sodium carbonate and 100μL IS to 1 mL of plasma, extract with 6 mL of MTBE. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve in 300 μL MeCN:10 mM perchloric acid 45:55, add 4 mL of hexane, vortex, centrifuge to separate the layers, freeze in a dry ice/acetone bath, discard the organic layer, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 23 × 4 5 μm YMC ODS-AQ
Column: 50 × 4 3 μm YMC ODS-AQ
Mobile phase: MeCN:MeOH:10 mM tetramethylammonium perchlorate in water:trifluoroacetic acid 40:5:55:0.1
Flow rate: 1.5
Injection volume: 100
Detector: UV 205

CHROMATOGRAM
Limit of quantitation: 5.6 ng/mL

OTHER SUBSTANCES
Extracted: ABT-378

KEY WORDS
plasma

REFERENCE
Bryan,P.; el-Shourbagy,T.; Emry,M.; Marsh,K.; McDonald,E.; Brooks,R.; Sapochak,L.; Hsu-Beischer,R.; Chu,S. A sensitive and specific HPLC method for the simultaneous determination of ABT-378 and ritonavir in human plasma using uv detection (Abstract 2646), *Pharm.Res.*, **1997**, *14*, S427.

SAMPLE
Matrix: blood, CSF, saliva
Sample preparation: Add 400 μL MeCN to 100 μL plasma, CSF, or saliva, vortex for 30 s, centrifuge at 10500 g for 3 min, evaporate 400 μL supernatant to dryness under a gentle stream of nitrogen at 40°, redissolve the residue in 150 μL mobile phase, vortex for 60 s, centrifuge at 10500 g for 3 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chromguard C18 (Chrompack, Netherlands)
Column: 75 × 4.6 3.5 μm Zorbax SB-C18
Mobile phase: MeCN:buffer 44:56 (Buffer was 25 mM sodium acetate with 25 mM hexane-1-sulfonic acid adjusted to pH 4.0 with 37% HCl.)
Flow rate: 1
Injection volume: 100
Detector: UV 239

CHROMATOGRAM
Retention time: 9
Limit of detection: 20 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: didanosine, fluconazole, folinic acid, ganciclovir, indinavir, lamivudine, methadone, methotrexate, nelfinavir, oxazepam, pyrazinamide, ranitidine, rifampin, saquinavir, stavudine, sulfamethoxazole, trimethoprim, zalcitabine, zidovudine, zidovudine glucuronide

KEY WORDS
plasma

REFERENCE
Hoetelmans,R.M.W.; van Essenberg,M.; Profijt,M.; Meenhorst,P.L.; Mulder,J.W.; Beijnen,J.H. High-performance liquid chromatographic determination of ritonavir in human plasma, cerebrospinal fluid and saliva, *J.Chromatogr.B*, **1998**, *705*, 119–126.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add four volumes of MeCN to microsomal incubation, centrifuge at 1500 g for 10 min, evaporate supernatant to dryness under nitrogen at 40°, resuspend the residue in 100-120 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5C18 (Phenomenex)
Mobile phase: Gradient. A was MeCN. B was 0.1% triethylamine in water adjusted to pH 4.8 with ammonium acetate. A:B from 25:75 to 75:25 over 15 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 210

CHROMATOGRAM
Retention time: 12.9

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE

Koudriakova,T.; Iatsimirskaia,E.; Utkin,I.; Gangl,E.; Vouros,P.; Storozhuk,E.; Orza,D.; Marinina,J.; Gerber,N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: Mechanism-based inactivation of cytochrome P4503A by ritonavir, *Drug Metab.Dispos.*, **1998**, *26*, 552–561.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

CHROMATOGRAM

Retention time: 22-27

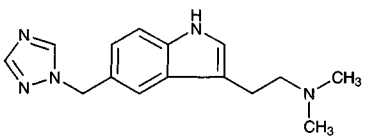
OTHER SUBSTANCES

Simultaneous: indinavir, nelfinavir, saquinavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

REFERENCE

Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

Rizatriptan



Molecular formula: $C_{15}H_{19}N_5$
Molecular weight: 269.35
CAS Registry No.: 144034-80-0, 145202-66-0 (benzoate), 59776-67-7 (sulfate)

SAMPLE

Matrix: bulk
Sample preparation: Prepare an 1 mg/mL solution of the free base in MeCN:water 10:90. Inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax SB-phenyl
Mobile phase: MeCN:0.1% trifluoroacetic acid in water 16: 84 (A) or MeCN:0.1% phosphoric acid in water 12:88 (B)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM

Retention time: 6.3 (A), 6.7 (B)

OTHER SUBSTANCES

Extracted: impurities

REFERENCE

Antonucci,V.; Wright,L.; Toma,P. The reversed-phase liquid chromatographic behavior of the new 5-HT1D receptor agonist rizatriptan benzoate and its potential process impurities, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1649–1670.

SAMPLE
Matrix: bulk
Sample preparation: Prepare an 1 mg/mL solution of the free base in MeCN:water 10:90. Inject an aliquot.

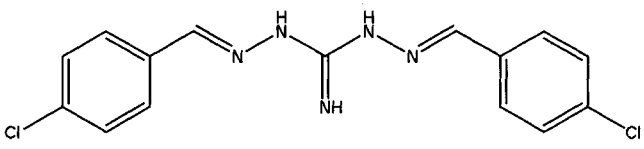
HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:0.1% trifluoroacetic acid in water 16: 84 (A) or MeCN:0.1% phosphoric acid in water 12:88 (B)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5 (A), 6.7 (B)

OTHER SUBSTANCES
Extracted: impurities

REFERENCE
Antonucci,V.; Wright,L.; Toma,P. The reversed-phase liquid chromatographic behavior of the new 5-HT1D receptor agonist rizatriptan benzoate and its potential process impurities, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1649–1670.

Robenidine



Molecular formula: $C_{15}H_{13}Cl_2N_5$
Molecular weight: 334.21
CAS Registry No.: 25875-51-8, 25875-50-7 (HCl)
Merck Index: 8403

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 200 μg/mL solution in dichloromethane:MeOH 90:10, evaporate an aliquot to dryness under a stream of nitrogen at 60°, reconstitute with 1 mL 4 mg/mL 4-dimethylaminopyridine in dichloromethane, add 1 mL 2 mg/mL dansyl chloride in dichloromethane, heat in a capped tube at 80° for 1 h, cool on ice, add to a 3 mL Sep-Pak silica SPE cartridge, rinse the tube with 5 mL dichloromethane, add the rinse to the SPE cartridge, elute with 5 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 150 × 3.2 7 μm silica (Brownlee)
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Hexane:chloroform:THF:MeOH 50:50:2:1
Flow rate: 2
Injection volume: 20
Detector: F ex 320 em 485

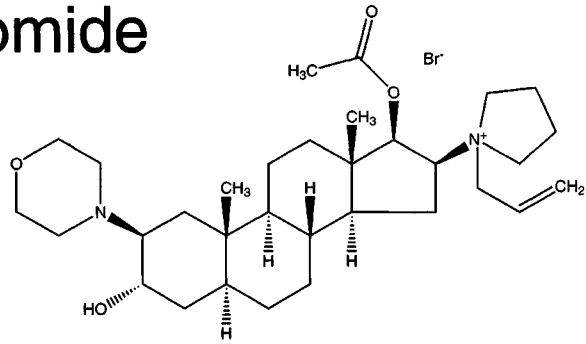
CHROMATOGRAM
Retention time: 4
Limit of detection: 400 ng/mL

KEY WORDS
derivatization; normal phase; protect from light; SPE

REFERENCE
Cohen,H.; Armstrong,F.; Campbell,H. Sensitive fluorescence detection of robenidine by derivatization with dansyl chloride and high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *694*, 407–413.

Rocuronium bromide

Molecular formula: $C_{32}H_{53}BrN_2O_4$
Molecular weight: 609.69
CAS Registry No.: 119302-91-9
Merck Index: 8407



SAMPLE
Matrix: bile, blood, stoma fluid, tissue, urine
Sample preparation: Homogenize (Ultra-Turrax) 1 g tissue with 9 mL 1 M NaH_2PO_4 for 10 min. Acidify 1 mL plasma, urine, or bile with 200 µL 1 M NaH_2PO_4. Homogenize 1 mL stoma fluid with 200 µL 1 M NaH_2PO_4. Make up 50-1000 µL plasma, 200-1000 µL urine, 5-200 µL bile, 1000 µL stoma fluid, or 100-1000 µL tissue homogenate to 2 mL with water, add 1 mL buffer, add 150 ng IS, add 7 mL dichloromethane, vortex for 15 s, centrifuge at 740 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot (or less). (Buffer was prepared by mixing 6 mL of an aqueous solution containing 7.505 mg/mL glycine and 5.85 mg/mL NaCl, 4 mL 100 mM NaOH and 6.2 g KI.)

HPLC VARIABLES
Guard column: 4 × 6 µBondapak C18
Column: 150 × 3.9 5 µm Lichrospher 100-RP18
Mobile phase: Dioxane:buffer 16:84 (Caution! Dioxane is a carcinogen!) (Buffer was 100 mM NaH_2PO_4 containing 0.11 mM 9,10-dimethoxyanthracene-2-sulfonate and 0.11 mM 1-heptanesulfonic acid, pH adjusted to 3.0 with orthophosphoric acid. After each series of analyses flush column with 15 mL water and 75 mL MeOH.)
Flow rate: 1
Injection volume: 100
Detector: F ex 385 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1 mL/min and the mixture flowed through a 1 m × 0.25 mm i.d. stainless steel coil to a phase separator (Organon International) then the organic phase flowed through the detector (J. Chromatogr. 1987, 421, 327; Anal. Chim. Acta 1987, 192, 267).

CHROMATOGRAM
Retention time: 9
Internal standard: 1-(3α,17β-dihydroxy-2β-morpholino-5α-androstan-16β-yl)-1-methylpiperidinium bromide (Org 7402, Organon) (21)
Limit of detection: 5 ng tissue, 4 ng (urine, bile), 3 ng (plasma)
Limit of quantitation: 20 ng/mL (stoma fluid), 100 ng/mL (bile), 25 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cephamandole, cephradine, dixyrazine, meperidine, metocurine, metoprolol, sulfamethoxazole, trimethoprim
Noninterfering: alfentanil, aprotinin, atropine, bupivacaine, chlorpromazine, daltaparin, dexamethasone, diazepam, dopamine, droperidol, etomidate, fentanyl, furosemide, gallamine, haloperidol, midazolam, morphine, neostigmine, nitroglycerin, nitroprusside, oxytocin, pancuron-

ium, pentobarbital, phenylephrine, phenytoin, pipecuronium, piperacillin, promethazine, propofol, ranitidine, succinylcholine, sufentanil, terbutaline, thiopental, vecuronium, verapamil
Interfering: alizapride, atracurium, ketamine, ketogan, lidocaine, metoclopramide, nimodipine, prochlorperazine, tubocurarine

KEY WORDS
human; dog; plasma; liver; lung

REFERENCE
Kleef,U.W.; Proost,J.H.; Roggeveld,J.; Wierda,J.M.K.H. Determination of rocuronium and its putative metabolites in body fluids and tissue homogenates, *J.Chromatogr.*, **1993**, *621*, 65–76.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH:MeCN 2:1 and 1 mL water. Acidify 1 mL plasma with 200 μL 1 M NaH_2PO_4. Add 20-200 ng IS to 1 mL acidified plasma, add to the SPE cartridge, wash with 1 mL water, wash with 1 mL 100 mM pH 3 NaH_2PO_4, elute with 400 μL mobile phase, discard first 100 μL eluate, inject a 200 μL aliquot of the remaining eluate (from J. Chromatogr. 1987, 421, 327; modifications may be necessary).

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Dioxane:water 20:80 containing 100 mM NaH_2PO_4 and 0.44 mM 9,10-dimethoxyanthracene-2-sulfonate, pH adjusted to 3 with phosphoric acid. (Caution! Dioxane is a carcinogen!) (After each series of analyses flush column with 200 mL MeOH then re-equilibrate with 120 mL mobile phase.)
Flow rate: 1
Injection volume: 200
Detector: F ex 380 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1.6 mL/min and the mixture flowed through a 1 m × 0.25 mm i.d. stainless steel coil to a phase separator (Anal. Chim. Acta 1987, 192, 267) then the organic phase flowed through the detector.

CHROMATOGRAM
Internal standard: 3,17-didesacetyl vecuronium (Org 7402)
Limit of quantitation: 10 ng/mL

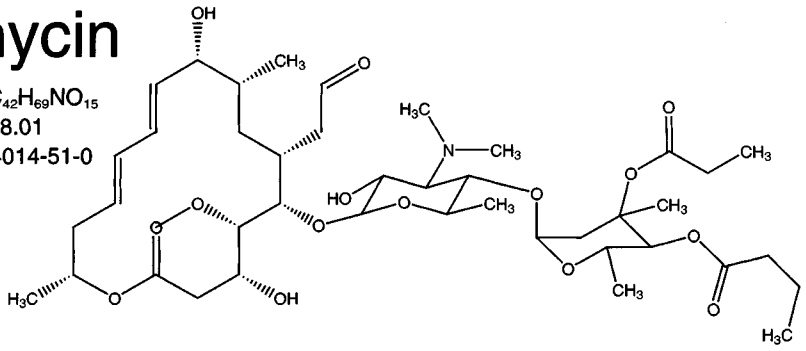
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics; post-column reaction; post-column extraction

REFERENCE
Cooper,R.A.; Maddineni,V.R.; Mirakhur,R.K.; Wierda,J.M.K.H.; Brady,M.; Fitzpatrick,K.T.J. Time course of neuromuscular effects and pharmacokinetics of rocuronium bromide (Org 9426) during isoflurane anaesthesia in patients with and without renal failure, *Br.J.Anaesth.*, **1993**, *71*, 222–226.

Rokitamycin

Molecular formula: $C_{42}H_{69}NO_{15}$
Molecular weight: 828.01
CAS Registry No.: 74014-51-0
Merck Index: 8408



SAMPLE

Matrix: blood

Sample preparation: Collect blood in tubes containing neostigmine methyl sulfate, final concentration 0.2 mM. 1 mL Plasma + 100 μL 2 μg/mL josamycin in MeOH, add 50 μL 100 mM pH 4.65 acetate buffer + 100 μL saturated NaCl + 100 μL 10 mM sodium lauryl sulfate + 5 mL hexane:isoamyl alcohol 90:10, shake for 15 min, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen with mild heating, reconstitute the residue in 200 μL 0.002% dansylhydrazine in toluene:MeOH:acetic acid 90:10:1.13 (freshly prepared), heat at 60° for 20 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute with 20 μL ethyl acetate, add 60 μL 100 mM HCl, inject a 20 μL aliquot of the lower aqueous phase. (Silanize glassware with 1% trimethylchlorosilane in toluene for 1 h at 70°, wash twice with MeOH.)

HPLC VARIABLES

Column: 150 × 4.6 3 μm Nucleosil C18
Mobile phase: MeCN:50 mM ammonium acetate 72:28
Column temperature: 32.5
Flow rate: 0.8
Injection volume: 20
Detector: F ex 352 em 537

CHROMATOGRAM

Retention time: 11.5
Internal standard: josamycin (9.5)
Limit of detection: 20 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

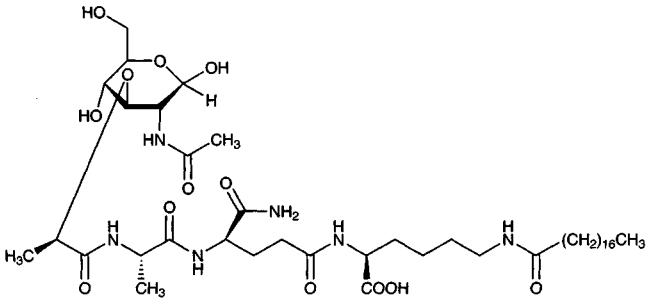
KEY WORDS

plasma; derivatization

REFERENCE

Tod,M.; Biarez,O.; Nicolas,P.; Petitjean,O. Sensitive determination of josamycin and rokitamycin in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1992**, *575*, 171–176.

Romurtide



Molecular formula: $C_{43}H_{78}N_6O_{13}$
Molecular weight: 887.12
CAS Registry No.: 78113-36-7
Merck Index: 8412

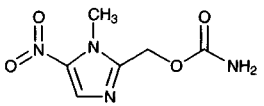
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm octadecyl silica
Mobile phase: MeCN:MeOH:20 mM ammonium acetate 66:4:40
Column temperature: 25
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8.6

REFERENCE
Moroi,R.; Yamazaki,K.; Hirota,T.; Watanabe,S.; Kataoka,K.; Ichinose,M. Physico-chemical properties of muroctasin, *Arzneimittelforschung*, **1988**, *38*, 953–959.

Ronidazole



Molecular formula: $C_6H_8N_4O_4$
Molecular weight: 200.15
CAS Registry No.: 7681-76-7
Merck Index: 8413
Lednicer No.: 2 245

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

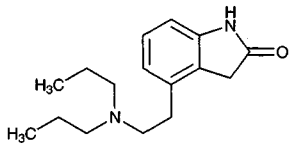
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 308

CHROMATOGRAM
Retention time: 8.265

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ropinirole



Molecular formula: $C_{16}H_{24}N_2O$
Molecular weight: 260.38
CAS Registry No.: 91374-21-9, 91374-20-8 (HCl)
Merck Index: 8416

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation + 50 μL 5% trichloroacetic acid, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ (Supelco)
Mobile phase: Gradient. A was MeCN. B was 100 mM pH 4 ammonium acetate. A:B from 0:100 to 15:85 over 10 min, to 100:0 over 12 min, maintain at 100:0 for 15 min
Column temperature: 40
Flow rate: 1
Detector: UV 250; Radioactivity, Ramona-5 (Lablogic, Inc., UK)

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
liver

REFERENCE
Bloomer,J.C.; Clarke,S.E.; Chenery,R.J. In vitro identification of the P450 enzymes responsible for the metabolism of ropinirole, *Drug Metab.Dispos.*, **1997**, *25*, 840–844.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

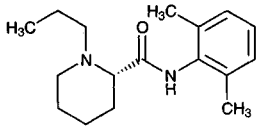
CHROMATOGRAM
Retention time: k' 0.95

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Ropivacaine



Molecular formula: $C_{17}H_{26}N_2O$
Molecular weight: 274.41
CAS Registry No.: 84057-95-4, 98717-15-8 (HCl), 132112-35-7 (HCl monohydrate)
Merck Index: 8417

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond Elut SCX SPE cartridge. Hydrolyze 1 mL urine with 6 M HCl in a water-bath at 95° for 1 hr, dilute with water 1:5. Acidify plasma with phosphoric acid. Add the sample to the SPE cartridge, wash with 200 mM pH 4.5 acetate buffer, wash with MeOH:buffer 50:50, elute with MeOH:2 M ammonia 80:20, evaporate to dryness, reconstitute with 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 2 Superspher RP-Select B (plasma) or 125 × 4 Superspher RP-Select B (urine)
Mobile phase: MeCN:pH 2 phosphate buffer 15-25:85-75 containing 5-15 mM octanesulfonic acid
Detector: UV 210

CHROMATOGRAM
Limit of quantitation: 10 ng/mL (plasma), 300 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Halldin,M.M.; Bredberg,E.; Angelin,B.; Arvidsson,T.; Askemark,Y.; Elofsson,S.; Widman,M. Metabolism and excretion of ropivacaine in humans, *Drug Metab.Dispos.*, **1996**, *24*, 962–968.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase to a ropivacaine hydrochloride concentration of 75 μg/mL, inject an aliquot.

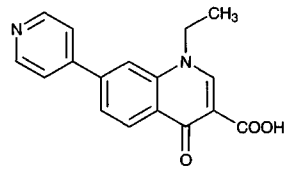
HPLC VARIABLES
Guard column: 10 × 3 chiral-AGP
Column: 100 × 4 5 μm chiral-AGP (Chromtech)
Mobile phase: Isopropanol:pH 7.2 phosphate buffer (μ = 0.05) 7:93
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 9 (R), 12 (S)

KEY WORDS
chiral; injections; comparison with capillary electrophoresis

REFERENCE
Sänger-van de Griend,C.E.; Wahlström,H.; Gröningsson,K.; Widahl-Näsman,M. A chiral capillary electrophoresis method for ropivacaine hydrochloride in pharmaceutical formulations: validation and comparison with chiral liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1051–1061.

Rosoxacin



Molecular formula: $C_{17}H_{14}N_2O_3$
Molecular weight: 294.31
CAS Registry No.: 40034-42-2
Merck Index: 8426
Lednicer No.: 3 185

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 5.13
Internal standard: sparfloxacin (8.3)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 271.2

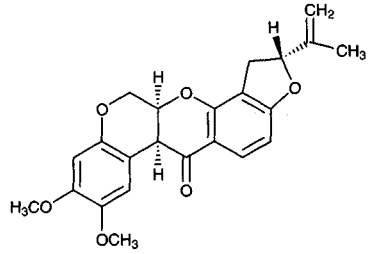
CHROMATOGRAM
Retention time: 13.467

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Rotenone

Molecular formula: $C_{23}H_{22}O_6$
Molecular weight: 394.42
CAS Registry No.: 83-79-4
Merck Index: 8427



SAMPLE
Matrix: feed
Sample preparation: 10 g Ground feed + 100 mL MeCN:glacial acetic acid 99:1, shake for 1 h, centrifuge, filter (0.45 μm) an aliquot of the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Simultaneous: rotenonone

REFERENCE
Kline,D.A.; Hanna,G.R.; Honaker,C.B.; Kuhn,G.O.; Jameson,C.W. Preparation and stability of animal feed mixtures dosed with rotenone, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 660–663.

SAMPLE
Matrix: sediment, tissue
Sample preparation: Fish, crayfish, mussels. Homogenize (Waring blender) with dry ice, place in freezer overnight. 10 g Sample + 60 (fish), 70 (fish offal, crayfish) or 100 (mussels) g anhydrous sodium sulfate, mix (Sorval Omni-mixer), place sample on top of 50 mm anhydrous sodium sulfate in a 22 mm diameter glass column, place a 50 mM layer of anhydrous sodium sulfate on top of the column, pass 100 mL diethyl ether through the column at 1.5 mL/min. Evaporate the eluate to dryness under reduced pressure at 30°, reconstitute the residue in 10 mL dichloromethane:cyclohexane 50:50, add a 5 mL aliquot to a 400 × 28 column packed with SX-3 BioBeads (Analytical BioChemistry Labs) in dichloromethane:cyclohexane 50:50, elute with dichloromethane:cyclohexane 50:50 at 4 mL/min, discard the first 112 mL of eluate, collect the second 112 mL and evaporate it to dryness under reduced pressure at 30°. Reconstitute with 5 mL benzene (Caution! Benzene is a carcinogen!) and add to the silica column, rinse flask with five 5 mL portions of benzene, add rinses to the column (do not allow column to go dry), elute with 70 mL benzene:acetone 97:3. Evaporate the eluate to dryness under reduced pressure at 30°, reconstitute in 5 mL MeOH, inject a 100 μL aliquot. Sediment. 10 g Sediment + 20 mL MeOH, mix (Sorval Omni-mixer), centrifuge at 1000 g for 5 min, repeat extraction 3 more times with 10 mL MeOH. Filter (Gelman type A/E glass fiber) the combined supernatants, evaporate the filtrate under reduced pressure at 30° to about 25 mL. Add the residue to 500 mL 100 mM HCl, extract three times with 20 mL hexane. Combine the hexane extracts and evaporate them to dryness under reduced pressure at 30°, reconstitute with 5 mL benzene (Caution! Benzene is a carcinogen!) and add to the silica column, rinse flask with five 5 mL portions of benzene, add rinses to the column (do not allow column to go dry), elute with 70 mL benzene:acetone 97:3. Evaporate the eluate to dryness under reduced pressure at 30°, reconstitute in 5 mL MeOH, inject a 100 μL aliquot. (Column was 5 g anhydrous sodium sulfate, 5 g 3% deactivated silica gel, and 5 g anhydrous sodium sulfate, all slurry packed in benzene. Silica gel was 40-140 mesh from J.T. Baker activated at 130° for 24 h.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:water 70:30 (60:40 for fish offal)
Flow rate: 1
Injection volume: 100
Detector: UV 295

CHROMATOGRAM
Retention time: 5 , 17 (for fish offal)
Limit of detection: 5 ng/g (tissue), 25 ng/g (sediment)

KEY WORDS
fish; crayfish; mussels; shellfish; sediment

REFERENCE
Dawson,V.K.; Allen,J.L. Liquid chromatographic determination of rotenone in fish, crayfish, mussels, and sediments, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1094–1096.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) water, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Zorbax ODS

Mobile phase: MeCN:water 70:30
Flow rate: 1.3
Injection volume: 200
Detector: UV 210

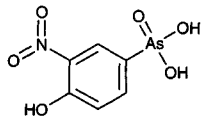
CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 7.5 ppb

OTHER SUBSTANCES
Simultaneous: rotenonone

KEY WORDS
drinking water; stream water; pond water; water

REFERENCE
Bushway,R.J. High-performance liquid chromatographic analysis of rotenone and rotenonone in water by direct injection, *J.Chromatogr.*, **1984**, *303*, 263–266.

Roxarsone



Molecular formula: $C_6H_6AsNO_6$
Molecular weight: 263.04
CAS Registry No.: 121-19-7
Merck Index: 8430

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass through No. 20 sieve. 15 g Ground feed + 50 mL 20 g/L K_2HPO_4, shake for 5 min, centrifuge at 2385 rpm for 10 min. REmove a 30 mL aliquot of the supernatant and add it to 1 mL 2.5 M HCl, let stand for 15 min, centrifuge for 10 min. Remove a 25 mL aliquot and add it to 1 mL 6 M NaOH, add 2 g activated charcoal (Darco G-60 or equivalent), swirl, let stand for 30 min with periodic swirling, filter (0.45 μm) an aliquot, add the filtrate to an activated carbon SPE cartridge (Analtech No. 01-97), discard the first 2 mL, inject a 15 μL aliquot of the next 500 μL of eluate.

HPLC VARIABLES
Guard column: Bondapak C18
Column: 8NVC18 Radial Pak radial compression (Waters)
Mobile phase: MeOH:water 25:75 containing 3% PIC-A (tetrabutylammonium sulfate)
Flow rate: 1.2
Injection volume: 15
Detector: UV 243

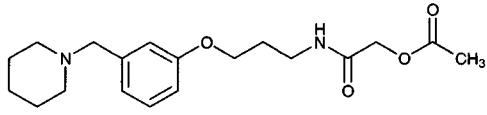
CHROMATOGRAM
Retention time: 8
Limit of detection: <0.3 ng

OTHER SUBSTANCES
Noninterfering: bacitracin, bambemycins, BHA, BHT, chlortetracycline, erythromycin, ethoxyquin, furazolidone, hygromycin B, monensin, niacin, nicarbazin, ormetoprim, oxytetracycline, riboflavin, sulfadimethoxine, tetracycline, thiamine, vitamin D3, vitamin E, pyridoxine, vitamin A

KEY WORDS
SPE

REFERENCE
Sapp,R.E.; Davidson,S. Determination of Roxarsone in feeds using solid phase extraction and liquid chromatography with ultraviolet detection, *J.AOAC Int.*, **1993**, *76*, 956–961.

Roxatidine acetate



Molecular formula: $C_{19}H_{28}N_2O_4$
Molecular weight: 348.44
CAS Registry No.: 78628-28-1, 93793-83-0 (HCl)
Merck Index: 8431
Lednicer No.: 5 26

SAMPLE
Matrix: solutions

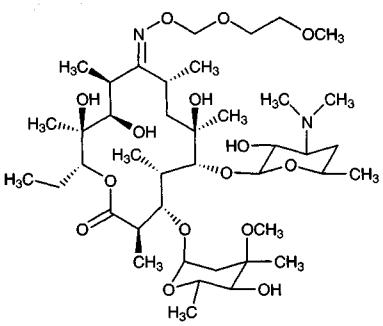
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.29

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Roxithromycin



Molecular formula: $C_{41}H_{76}N_2O_{15}$
Molecular weight: 837.06
CAS Registry No.: 80214-83-1
Merck Index: 8433

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 20 µg/mL IS in MeOH + 2.5 mL hexane:isoamyl alcohol 95:5, vortex for 20 s, centrifuge at 1500 g for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 µL mobile phase, vortex for 20 s, sonicate for 1 min, centrifuge at 1500 g for 5 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 mm long Supelguard C18 (Supelco)
Column: 50 × 4.6 5 µm Supelcosil C18
Mobile phase: MeCN:MeOH:water 50:20:30 containing 4 g/L (?) ammonium acetate
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem 5100A, 5010 analytical cell, +0.89 V

CHROMATOGRAM

Retention time: 3.5
Internal standard: RU 29767 (7.5)
Limit of detection: 50 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Nilsen,O.G.; Aamo,T.; Zahlsen,K.; Svarva,P. Macrolide pharmacokinetics and dose scheduling of roxithromycin, *Diagn.Microbiol.Infect.Dis.*, **1992**, *15*, 71S–76S.

SAMPLE

Matrix: blood
Sample preparation: Condition a 10 × 2 20 mg 30-40 µm Baker CN SPE cartridge with 2 mL MeOH, 2 mL MeOH:water 10:90, and 4 mL MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90 at 2 mL/min. Centrifuge plasma at 1300 g for 5 min, 100 µL plasma + 100 µL clarithromycin in MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90, mix, add a 20-100 µL aliquot to the SPE cartridge, wash SPE cartridge with MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90 at 0.5 mL/min, after 5 min backflush the contents of the SPE cartridge onto the column with the mobile phase, elute the column with the mobile phase and monitor the effluent.

HPLC VARIABLES

Column: 100 × 4.6 3 µm Hypersil BDS C18
Mobile phase: MeCN:water 54:46 containing 4.5 mM NaH_2PO_4 and 6.8 mM Na_2HPO_4, pH 7
Column temperature: 55
Flow rate: 1
Detector: E, ESA Coulochem II, Model 5011 dual analytical cell, upstream +0.65 V, downstream +0.85 V (monitored), analytical cell protected by an ESA carbon in-line filter

CHROMATOGRAM

Retention time: 7

Internal standard: clarithromycin (6)
Limit of quantitation: 500 nM

KEY WORDS
plasma; SPE

REFERENCE
Hedenmo,M.; Eriksson,B.-M. Liquid chromatographic determination of the macrolide antibiotics roxithromycin and clarithromycin in plasma by automated solid-phase extraction and electrochemical detection, *J.Chromatogr.A*, **1995**, *692*, 161–166.

SAMPLE
Matrix: blood, gastric juice, gastric mucosa, saliva, vitreous humor
Sample preparation: Homogenize 5-20 mg gastric mucosa in 300 μL 10 mM pH 7.4 sodium phosphate buffer with sonication. Add 500 ng clarithromycin in MeOH:water 50:50 to 500 μL plasma, serum, saliva, gastric juice, leucocytes lysate, vitreous humor or 300 μL gastric mucosa homogenate, vortex, add 200 μL 100 mM sodium carbonate and 3 mL MTBE, shake thoroughly (5 × 2 s in an SMI Multi-tube vortexer), centrifuge at 1000 g for 5 min, freeze the aqueous layer in liquid nitrogen or in a freezer at -70° for 15 min. Evaporate the upper organic layer to dryness in a centrifugal vacuum evaporator (Jouan RC 10.22), reconstitute the residue in 250 μL MeOH:water 50:50, inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB CN
Mobile phase: MeCN:MeOH:50 mM Na_2HPO_4 and NaH_2PO_4 buffer 53:41:6 (pH 7.0) (The mobile phase was a mixture of 350 mL MeCN, 50 mL MeOH and 450 mL 50 mM Na_2HPO_4 and NaH_2PO_4 buffer.)
Column temperature: 30
Flow rate: 1
Injection volume: 20-50
Detector: E, ESA Coulochem II, guard cell +1.0 V, screening cell E1 +0.50 V, analytical cell E2 +0.80 V

CHROMATOGRAM
Retention time: 11.5
Internal standard: clarithromycin (10)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: azithromycin

KEY WORDS
pharmacokinetics; plasma; saliva; serum; leucocytes

REFERENCE
Kees,F.; Spangler,S.; Wellenhofer,M. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 287–293.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: Plasma. 2 mL Plasma + 20 μL 750 μg/mL roxithromycin in MeCN + 5 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 5 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot. Urine. 1.5 mL Urine + 100 μL 750 μg/mL roxithromycin in saturated K_2HPO_4 + 4 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 5 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot. Saliva. 1.5 mL Saliva + 100 μL 750 μg/mL roxithromycin in saturated K_2HPO_4 + 4 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 15 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot.

HPLC VARIABLES
Column: Nova-Pak C18
Mobile phase: MeCN:MeOH:56 mM sodium acetate buffer 50:4:56, final pH adjusted to 7.0 with glacial acetic acid
Flow rate: 1.1
Injection volume: 40
Detector: E, Waters M460, +0.9 V versus Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 14.7
Internal standard: roxithromycin
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Simultaneous: 4″-acetylerythromycin, 6-O-methylerythromycin, erythromycin base, erythromycin B, erythromycin estolate, erythromycin ethylsuccinate

KEY WORDS
plasma; roxithromycin is IS

REFERENCE
Croteau,D.; Vallée,F.; Bergeron,M.G.; LeBel,M. High-performance liquid chromatographic assay of erythromycin and its esters using electrochemical detection, *J.Chromatogr.*, **1987**, *419*, 205–212.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 1:2 with isotonic NaCl. 200 μL Plasma or diluted urine + 100 μL 10 μg/mL erythromycin in water + 600 μL pH 9 phosphate buffer + 3 mL dichloromethane, shake for 10 min, centrifuge at 2000 g for 5 min. Remove 2.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, vortex for 10 s, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:83 mM ammonium acetate 55:22:23, pH adjusted to 7.5 with acetic acid
Flow rate: 1
Injection volume: 15
Detector: E, ESA Coulochem Model 5100A, Model 5020 guard cell 1.0 V (before injector), Model 5010 dual-electrode cell, screen electrode E1 + 0.7 V, sample electrode E2 +0.9 V, 0.5 μm ESA carbon filters placed before guard and analytical cells

CHROMATOGRAM
Retention time: 9.8
Internal standard: erythromycin (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, clomipramine, disopyramide, erythromycin estolate, erythromycin ethylsuccinate, erythromycin stearate, imipramine, josamycin, lidocaine, spiramycin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Demotes-Mainaird,F.M.; Vinçon,G.A.; Jarry,C.H.; Albin,H.C. Micro-method for the determination of roxithromycin in human plasma and urine by high-performance liquid chromatography using electrochemical detection, *J.Chromatogr.*, **1989**, *490*, 115–123.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.833

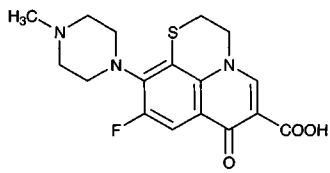
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Rufloxacin



Molecular formula: $C_{17}H_{18}FN_3O_3S$
Molecular weight: 363.41
CAS Registry No.: 101363-10-4
Merck Index: 8448

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma. 500 µL Plasma + 25 µL 350 µg/mL pipemidic acid in water + 50 µL 70% perchloric acid, vortex for 20 s, centrifuge at 14926 g for 5 min, inject a 10 µL aliquot of the supernatant. Urine. 500 µL Urine + 500 µL water + 1 mL 35 µg/mL pipemidic acid in water + 1.5 g Na_2HPO_4:NaH_2PO_4 50:50, mix, add 8 mL dichloromethane, agitate horizontally at low sped for 40 min, centrifuge at 6590 g for 5 min. Remove 5 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 10 µL aliquot. Alternatively, dilute 1 mL urine to 50 mL with water, inject a 10 µL aliquot. Bile. Centrifuge 500 µL bile at 14926 g for 15 min, filter (0.2 µm) the supernatant, inject a 5 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 3.2 7 µm Polymer RP (Brownlee)
Column: 50 × 4.1 10 µm PRP1 poly(styrene-divinylbenzene) (Hamilton)
Mobile phase: MeCN:0.17% phosphoric acid 12:88, adjust to pH 5.6 with triethylamine then add 5 mL/L THF
Flow rate: 1
Injection volume: 5-10
Detector: F ex 350 em 510 or UV 300

CHROMATOGRAM
Retention time: 8.9
Internal standard: pipemidic acid (3.5)
Limit of detection: 10 ng/mL (F)
Limit of quantitation: 50 μg/mL (F)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; protect from light

REFERENCE
Lombardi,F.; Ardemagni,R.; Colzani,V.; Visconti,M. High-performance liquid chromatographic determination of rufloxacin and its main active metabolite in biological fluids, *J.Chromatogr.*, **1992**, *576*, 129–134.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 μL 100 μg/mL IS in 20 mM NaOH. Add 500 μL 50 mM pH 7.0 phosphate buffer, vortex for 1 min, add 2 mL dichloromethane, vortex for 1 min, shake for 5 min. Centrifuge at 100 g for 10 min, separate the organic layer, repeat the extraction procedure twice, evaporate the combined organic lowers to dryness under reduced pressure. Reconstitute with 200 μL 20 mM NaOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Supelcosil LC-SAX
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.3
Internal standard: fenbufen (3.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: furprofen

KEY WORDS
SPE; plasma

REFERENCE
Carlucci,G.; Mazzeo,P. Simultaneous determination of furprofen and rufloxacin in human plasma by high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1996**, *34*, 182–184.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate..

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 19:81 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 248

CHROMATOGRAM
Retention time: 4.68
Internal standard: enrofloxacin (7.53)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 2 mg/mL IS in MeOH, mix, add 2 mL dichloromethane:diethyl ether 80:20, vortex for 15 s, centrifuge at 1500 g for 5 min, remove a 1.7 mL aliquot of the organic phase, repeat the extraction twice more with 2 mL portions of dichloromethane:diethyl ether 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pelliguard C18 (Supelco)
Column: 250 × 4.6 5 μm Viosfer C18 (Violet, Rome)
Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.2
Internal standard: 2-[4-(2'-furoyl)phenyl]propionic acid (7.3)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: theophylline

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Mazzeo,P.; Palumbo,G. Simultaneous determination of rufloxacin and theophylline by high-performance liquid chromatography in human plasma, *Analyst*, **1995**, *120*, 2493–2495.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum + 1 mL 100 mM pH 7 phosphate buffer + 100 (serum) or 150 (urine) μL 5 μg/mL ofloxacin in 500 mM NaOH, mix, add 2.5 mL dichloromethane, shake for 10 min, centrifuge at 1500 g for 10 min, repeat the extraction twice. Combine the organic layers and evaporate them to dryness with nitrogen under vacuum, reconstitute the residue in 200 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm AXGU anion-exchange (Rainin)
Column: 250 × 4.6 10 μm anion-exchange (Vydac)
Mobile phase: MeCN:50 mM pH 7 phosphate buffer 20:80
Flow rate: 1.8
Injection volume: 20
Detector: UV 296

CHROMATOGRAM
Retention time: 7.5
Internal standard: ofloxacin (5.6)
Limit of detection: 50 ng/mL (urine), 100 ng/mL (serum)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Carlucci,G.; Palumbo,G. Analytical procedure for the determination of rufloxacin, a new pyridobenzothiazine, in human serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *564*, 346–351.

SAMPLE
Matrix: urine
Sample preparation: Dilute ten times with water, inject a 20 μL aliquot. Deconjugate by heating 100 μL urine, 100 μL 50000 U/mL β-glucuronidase (Type II from limpets Patella vulgata, Sigma), and 800 μL pH 3.8 KH_2PO_4 buffer at 37° for 16 h.

HPLC VARIABLES
Guard column: 75 × 2.1 10 μm pellicular reversed phase (Chrompack)
Column: 250 × 4.6 5 μm CP Spher 5-ODS (Chrompack)
Mobile phase: Gradient. MeCN:buffer from 4:96 to 26:74 over 37 min, return to initial conditions over 5 min. (Buffer was 6.75 mL orthophosphoric acid and 2 mL diethylamine in 1 L water, pH 3.1.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 246

CHROMATOGRAM
Retention time: 22
Limit of detection: 500 ng/mL

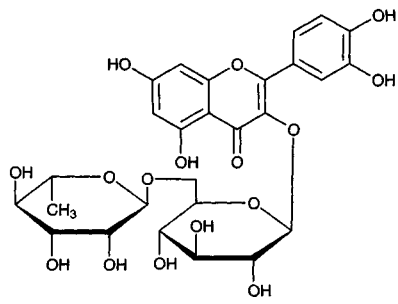
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
monkey; pharmacokinetics

REFERENCE
Vree,T.B.; Van den Biggelaar-Martea,M.; Peeters,A.; Imbimbo,B.P. High-performance liquid chromatography and preliminary pharmacokinetics of rufloxacin and its metabolites, N-desmethylrufloxacin and rufloxacin-sulfoxide, in urine of rhesus monkey *Macaca mulatta*, *J.Chromatogr.*, **1992**, *573*, 168–172.

Rutin

Molecular formula: $C_{27}H_{30}O_{16}$
Molecular weight: 610.53
CAS Registry No.: 153-18-4
Merck Index: 8456



SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

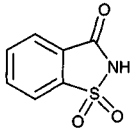
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 10.1

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Saccharin



Molecular formula: $C_7H_5NO_3S$
Molecular weight: 183.19
CAS Registry No.: 81-07-2, 6485-34-3 (Ca salt), 6381-91-5 (Ca salt hydrate), 128-44-9 (Na salt), 6155-57-3 (Na salt dihydrate)
Merck Index: 8463

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: benzoic acid, hydroquinine, quinine